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(54) Title: METHOD AND APPARATUS FOR DETERMINING THE EXISTENCE OF A MUTATION

(57) Abstract

In a method for determining the existence of a mutation in a nucleic acid fragment from an electric signal generated by a DNA sequencer and made up of sequence information produced by fluorescent fragment products of different lengths of said nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment, any difference between said run-off peak and a reference run-off peak generated by unmutated full length fragment products of said nucleic acid fragment, is determined, a difference indicating the existence of a deletion or insertion mutation.

METHOD AND APPARATUS FOR DETEPMINING THE EXISTENCE OF A MUTATION

TECHNICAL FIELD

The invention relates to a method and an apparatus for determining the existence of a mutation in a nucleic acid fragment from an electric signal generated by a DNA sequencer and made up of sequence information produced by fluorescent fragment products of different lengths of said nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment.

BACKGROUND OF THE INVENTION

DNA sequencing, i.e. determining the sequence of nucleotides in a gene or in a segment of DNA, commonly involves several sequential steps aimed at:

- Isolating genetic material from biological material.

- Amplifying the gene of interest using polymerase chain reaction (PCR) so that sufficient material of the gene of interest is available for sequence analysis.

- Performing sequencing reactions using the principles of Sanger. This step enzymatically generates a large number of differently elongated complementary copies of the gene. By introduction of base specific elongation terminators, each elongated copy of the gene will terminate with a specific type of nucleotide. Each reaction corresponds to one specific type of nucleotide, Adenine (A), Thymine (T), Guanine (G) or Cytosine (C), i.e. only one type of elongation terminator, will be incorporated in each reaction. To enable detection of these elongated gene copies, a fluorescently labelled molecule is introduced in the gene copy during the enzymatic reaction. Thus, all elongated gene copies will be fluorescently labelled to facilitate their detection.

- Separating the mixture of differently elongated complementary copies of the gene according to their molecular size using gel electrophoresis.

- Sequentially detecting the differently elongated complemen-

mixed up with an equally large amount of non-mutated material. In some cases, the non-mutated material will even be predominant. In these cases ordinary alignment algorithms fail to resolve the mutation.

BRIEF DESCRIPTION OF THE INVENTION

The object of the invention is to bring about a simple and reliable method of determining the existance of a mutation in a nucleic acid fragment.

In the method according to the invention for determining the existence of a mutation in a nucleic acid fragment from an electric signal generated by a DNA sequencer and made up of sequence information produced by fluorescent fragment products of different lengths of said nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment, this is attained, mainly, by determining any difference between said run-off peak and a reference run-off peak generated by unmutated full length fragment products of the nucleic acid fragment, a difference indicating the existence of a deletion or insertion mutation.

This object is also attained by the apparatus according to the invention for determining the existence of a mutation in a nucleic acid fragment from an electric signal generated by a DNA sequencer and made up of sequence information produced by fluorescent fragment products of different lengths of said nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment, mainly, in that it comprises means for determining any difference between said run-off peak and a reference run-off peak generated by unmutated full length fragment products of said nucleic acid fragment, a difference indicating the existence of a deletion or insertion mutation.

BRIEF DESCRIPTION OF THE DRAWING

The invention will be described more in detail below with reference to the appended drawing on which

from Fig. 2b, the normal "unmutated" run-off peak of Fig. 2a has been broadened, so that the run-off peak of Fig. 2b ends after the run-off peak of Fig. 2a.

In case of a large deletion mutation, a split run-off peak will be generated as shown in Fig. 2c. As apparent from Fig. 2c, the normal "unmutated" run-off peak coincides with the normal "unmutated" run-off peak of Fig. 2a, while the run-off peak from the fragments in which a large deletion mutation is present, will appear before the normal "unmutated" run-off peak.

The indication of insertion and deletion mutations based on the run-off peak behaviour, is very sensitive and a contribution of less than 5% mutated material can be readily detected. The resolution in size difference between the two fragments is however limited to about ± 2 bases. This is of course dependent on the resolution of the electrophoresis gel.

It should be understood, however, that when using the run-off peak information alone for the mutation assignment, no information about the localization of the mutation along the DNA fragment will be achievable.

The run-off peak information is a sensitive means for the mutation detection and may be used as a consistency check of the mutation assignment derived from sequence data.

In accordance with the invention, any difference between a run-off peak generated by mutated full length fragment products, such as the run-off peak shown in Fig. 2b, and a normal "unmutated" run-off peak or reference run-off peak generated by unmutated full length fragment products, such as the run-off peak shown in Fig. 2a, is determined as an indication of the existence of a mutation.

In accordance with a first embodiment of the method according to the invention, the difference between the peak width of the run-off peak in Fig. 2b and the peak width of the run-off

ment products of different lengths of said nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment, comprises means (not shown) for determining any difference between said run-off peak and a reference run-off peak generated by unmutated full length fragment products of said nucleic acid fragment, a difference indicating the existence of a deletion or insertion mutation.

In a first embodiment of the apparatus according to the invention, the means (not shown) for determining any difference between said run-off peak and said reference run-off peak, is adapted to measure any difference in peak width between these run-off peaks, a wider run-off peak ending after said reference run-off peak, indicating an insertion mutation, while a wider run-off peak beginning before said reference run-off peak indicating a deletion mutation, the size of the peak width difference being directly proportional to the size of the mutation.

In a second embodiment of the apparatus according to the invention, the means (not shown) for determining any difference between said run-off peak and said reference run-off peak, is adapted tp measure any difference between the location of the centres of these run-off peaks, a location of the centre of said run-off peak in front of the centre of said reference run-off peak indicating a deletion mutation, a location of the centre of said run-off peak after the centre of said reference run-off peak indicating an insertion mutation, the size of said difference being directly proportional to the size of the mutation.

The apparatus according to the invention is preferably implemented in computer software.

nucleic acid fragment, followed by a run-off peak produced by fluorescent full length fragment products of said nucleic acid fragment, **characterized in** that it comprises means for determining any difference between the run-off peak and a reference run-off peak generated by unmutated full length fragment products of said nucleic acid fragment, a difference indicating the existence of a deletion or insertion mutation.

5. Apparatus according to claim 4, **characterized in** that said means for determining any difference between said run-off peak and said reference run-off peak, is adapted to measure any difference in peak width between these run-off peaks, a wider run-off peak ending after said reference run-off peak, indicating an insertion mutation, while a wider run-off peak beginning before said reference run-off peak indicating a deletion mutation, the size of the peak width difference being directly proportional to the size of the mutation.

6. Apparatus according to claim 4, **characterized in** that said means for determining any difference between said run-off peak and said reference run-off peak, is adapted to measure any difference between the location of the centres of these run-off peaks, a location of the centre of said run-off peak in front of the centre of said reference run-off peak indicating a deletion mutation, a location of the centre of said run-off peak after the centre of said reference run-off peak indicating an insertion mutation, the size of said difference being directly proportional to the size of the mutation.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/68 // G01N 21/64
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EDOC, WPI, FULLTEXT, MEDLINE, BIOSIS, DBA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 4100279 A1 (HITACHI, LTD.), 18 July 1991 (18.07.91) --	1-6
A	EP 0648844 A2 (HITACHI ELECTRONICS ENGINEERING CO., LTD.), 19 April 1995 (19.04.95) --	1-6

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

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